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Investigations on genetic diversity of northern snakehead (*Channa argus*) populations in China using amplified fragment length polymorphism (AFLP) markers

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ABSTRACT

Genetic diversity of northern snakehead (*Channa argus*) from China were investigated using amplified fragment length polymorphism (AFLP) markers. A total of 361 loci were detected by seven AFLP selective primer combinations from 240 individuals collected at eight locations. The proportion of polymorphic loci and Nei's genetic diversity among populations ranged from 32.1 to 46.1% and 0.092 to 0.128, respectively. Compared to other fish species, the northern snakehead population exhibited a lower level of genetic diversity. Genetic differentiation (F_{st}) and genetic distance among populations ranged from 0.034 to 0.382 and from 0.015 to 0.155, respectively. Molecular variance analysis showed that there was significant differentiation ($p<0.05$) among eight populations. Results from UPGMA analysis indicated that the eight populations could be divided into four major clusters. These results would be useful for the conservation of the northern snakehead resources and in selective breeding programmes.

Keywords: AFLP, *Channa argus*, Genetic diversity, Population structure

Introduction

The northern snakehead *Channa argus* (Perciformes, Channoidei, Channidae) is widely distributed in China (Cheng and Zheng, 1987; Courtenay and Williams, 2004). It is a popular food fish owing to its good taste, high protein content and presence of very few intramuscular spine bones (Nie *et al*., 2002) as well as for its medical properties (Liu *et al*., 2000). People prefer to consume wild fish as compared to cultured ones in China. This made the wild northern snakehead more expensive than the cultured ones, and more and more wild northern snakeheads were caught from rivers and lakes, which resulted in the reduction in wild northern snakehead populations.

Information on genetic diversity of fish species is essential for conserving genetic resources as well as in restraining genetic decline (Li *et al*., 2007; Peng *et al*., 2009). Basic knowledge on genetic diversity and population structure of wild northern snakehead is less known in China. Previous studies focused primarily on its propagation (Gui, 2008; Landis *et al*., 2011), cultivation (Huang, 2011), embryology, development (Yue *et al*., 1998), haematology (Cheng *et al*., 2006) and nutrition (Nie *et al*., 2002). Amplified fragment length polymorphism (AFLP) marker is an effective PCR-based nuclear DNA fingerprinting technique for revealing genetic diversity and differentiation within and among populations (Liu and Cordes, 2004; Bensch and Akesson, 2005). It does not require any prior sequence information of the species under investigation and has been extensively used for genetic variation and differentiation studies in fish species (Wang *et al*., 2002; Congiu *et al*., 2002; Zhang *et al*., 2004; Ludwig, 2008; Gwo *et al*., 2008; Adiputra *et al*., 2012). Therefore in the present study, AFLP markers were applied to evaluate the genetic diversity of eight wild northern snakehead populations in China. Our aims were to elucidate the molecular genetic diversity and the genetic relationships among the populations.

Materials and methods

Sample collection and DNA extraction

A total of 240 northern snakehead (*Channa argus*) individuals were sampled from eight local populations in China. The sampling locations were: Caohu Lake of Caohu City in Anhui Province (CHL, n=30), Dongting Lake of Yueyang City in Hunan Province (DTL, n=30), Erhai Lake of Dali City in Yunnan Province (THL, n=30), Heilong River of Heihe City in Heilongjiang Province (HLR, n=30), Hongze Lake of Hongze County in Jiangsu Province (HZL, n=30), Poyang Lake of Nanchang City in Jiangxi Province (PYL, n=30), Tailu Lake of Changxing County in Zhejiang Province (THL, n=30) and Weishan Lake of Weishan County in Shandong Province (WSL, n=30). All these samples were collected in 2010. Total genomic DNA was extracted from the caudal fin using a standard extraction kit (DNeasy tissue kit, Baitaike Biotech Co., Ltd, China).

AFLP analysis

Procedure of AFLP analysis was based on Vos *et al*. (1995). Briefly, 100 ng of DNA was digested with double restriction enzyme (EcoRI/MseI) (New England Biolabs Co., Ltd., Beijing) and were ligated to two adapters (EcoRI-adapter/MseI- adapter) (Table 1) with T4 DNA Ligase (New England biolabs Co., Ltd., Beijing). One microliter of the ligated product was used for preamplification by PCR with adapter-homologous primers with one selective nucleotide. The PCR amplification profile was as follows: 3 min at 94°C, followed by 20 cycles of 30 s at 94°C, 30 s at 53°C and 50 s at 72°C.

The pre-amplification product was diluted 10-fold and was subjected to selective amplification using primers with three selective nucleotides, and seven pairs of selective AFLP primers (Table 1) were chosen and labeled with FAM fluorescence [the forward primer was 5′end-labelled with FAM, dyes (Shanghai Shenggong Biological Engineering & Technology and Service Co. Ltd., Shanghai, China)]. The selective amplification reaction was performed with touchdown PCR begun at 94°C for 3 min, followed by 13 cycles of 30 sec at 94°C, 30 sec at 65°C (decreasing by 0.7°C each cycle) for 30 s, 1 min at 72°C. The last reaction was performed at 94°C for 30 s, 55°C for 30 s; 72°C for 1 min with 25 cycles and final extension of 72°C for 5 min. Amplified product was sent to Meiji Biotech Co., Ltd for ABI 3730 high performance capillary electrophoresis.

Data analysis

For each individual, the DNA fingerprints were scored by apparatus inspection (ABI 3730), and the files were analysed with Genemarker V2.20. Polymorphic DNA bands were scored as, present (1) or absent (0) by the demarcation of signal strength 500 for each DNA sample, ranging from 50-500 bp.

Popgene version 3.2 software (Raymond and Rousset, 1995) was used to estimate Nei′s genetic diversity index (H), number of polymorphic site and genetic similarity. Hierarchical partition of genetic diversity was evaluated by analysis of molecular variance (AMOVA) using the program Arlequin 3.0 (Excoffier *et al*., 2005). The software was also employed to calculate pairwise F_{st} values and testing their significance by bootstrapping analysis (1000 replicates) for evaluating genetic differentiation between populations.

The genetic distance between the populations was calculated using the Popgene version 3.2 (Raymond and Rousset, 1995). The dendrogram of eight populations was constructed using unweighted pair-group method with arithmetic means (UPGMA) in MEGA 4.1 based on the genetic distance (Tamura *et al*., 2007).

Results

Population genetic variation of northern snakehead

The proportions of polymorphic loci provide a good measure of genetic variations within and among populations of northern snakehead. A total of 361 loci were detected in eight populations by the seven AFLP primer pairs, of which 268 (74.24%) were polymorphic (Table 2). The average number of total loci scored per primer pair was 51.57, ranging from 38 to 63 loci. The average number of polymorphic loci scored per primer was 38.28 loci, ranging from 28 to 50 loci (Table 2). The

Table 1. The adaptor sequences and selective amplification primers used in AFLP analysis

Primer pairs	Sequences
EcoRI-adapter	5′-CTCGTAGACTGCGTACC-3′/5′-AATTGGTACGCAGTCTAC-3′
MseI-adapter	5′-GACGATGAGTCCTGAG-3′/5′-TACTCAGGACTCAT -3′
Pre-Pe/Pre-Pm	5′-GACTGCGTACCAATTCA-3′/5′-GATGAGTCCTGAGTAAC-3′
E-ACC /M-CAG	5′-GACTGCGTACCAATTCACC-3′/5′-GATGAGTCCTGAGTAACAG-3′
E-AGG/M-CAC	5′-GACTGCGTACCAATTCAGG-3′/5′-GATGAGTCCTGAGTAACAC-3′
E-AGG/M-CAA	5′-GACTGCGTACCAATTCAGG-3′/5′-GATGAGTCCTGAGTAACAA-3′
E-ACC/M-CAA	5′-GACTGCGTACCAATTCACC-3′/5′-GATGAGTCCTGAGTAACAA-3′
E-ATC/M-CAA	5′-GACTGCGTACCAATTCATC-3′/5′-GATGAGTCCTGAGTAACAA-3′
E-ACC/M-CAA	5′-GACTGCGTACCAATTCACC-3′/5′-GATGAGTCCTGAGTAACAA-3′
E-AGG/M-CTA	5′-GACTGCGTACCAATTCAGG-3′/5′-GATGAGTCCTGAGTAACTA-3′

Table 2. The amplification results per AFLP primer pair of northern snakehead populations

Population	Number of total loci	Number of polymorphic loci	Proportion of polymorphic loci
E-ACC/M-CAG	47	31	65.96
E-AGG/M-CAC	38	28	73.68
E-AGG/M-CAA	43	30	69.77
E-ACC/M-CAA	57	46	80.70
E-ATC/M-CAA	63	43	68.25
E-ACC/M-CAA	61	50	81.97
E-AGG/M-CTA	52	40	76.92
Total	361	268	74.24

proportion of the polymorphic loci scored per primer was 73.89%, ranging from 65.96 to 81.97% (Table 2). Number of the amplification loci and proportion of the polymorphic loci were both large, which suggest that the AFLP marker provides abundant genetic information and it was a perfect genetic marker for population genetic analysis.

The eight northern snakehead populations examined here exhibited varying genetic diversity. The population with the highest proportion of polymorphic loci (46.08%) was that of the Weishan Lake (WSL), whereas that with the lowest proportion of polymorphic loci (32.09%) was of Erhai Lake (EHL) (Table 2). The average number of polymorphic loci was estimated as 116.88, ranging from 93 in the Heilong River (HLR) population to 141 in the Weishan Lake (WSL) population. The average Nei′s genetic diversity of the eight populations was estimated as 0.108. The population with the highest Nei′s genetic diversity (0.128) was Dongting Lake population (DTL), whereas that with the lowest was Heilong River population (HLR) in which the highest Nei′s genetic diversity was 0.092 (Table 3).

Population structure and differentiation of northern snakehead

Results of AMOVA showed that majority of the variance (80.58%) resulted from intra-population variations, and only 19.42% of the total variance was from inter-population differentiation. Overall genetic differentiation among northern snakehead from the eight populations was highly significant ($p<0.05$) (Table 4). Moreover, all pairwise F_{st} statistic values estimated among populations were also significant ($p<0.05$), suggesting that all eight populations were significantly different from each other (Table 4). These analyses indicated that several distinct populations of the northern snakehead existed in Chinese waters. The highest genetic difference among populations existed in the Heilong River population and Erhai Lake population (F_{st} = 0.382), whereas the lowest genetic difference was between the Weishan Lake population and Hongze Lake population (F_{st} = 0.0186).

The genetic distance suggested that populations in Weishan Lake population (WSL) and Hongze Lake population (HZL) were most genetically similar among the eight populations (Table 5), whereas the populations from Heilong River (HLR) and Erhai Lake (EHL) were most different genetically, whose genetic distance was 0.155 (Table 4).

Based on the genetic distance, the UPGMA dendrogram displayed four major clusters (Fig. 1). The Erhai Lake population and the Heilong River population formed basal clusters A and B, respectively. The remaining populations formed distinct clusters C and D. The cluster C contained the Weishan Lake and Hongze Lake populations. The cluster D contained the Taihu Lake, Caohu Lake, Dongting Lake and Poyang Lake populations (Fig. 1).

Table 3. Comparison of total loci and polymorphic loci among northern snakehead populations

Population	Number of total loci	Number of polymorphic loci	Proportion of polymorphic loci
CHL	296	118	0.399
DTL	301	133	0.442
EHL	296	95	0.321
HLR	286	93	0.325
HZL	300	129	0.430
PYL	298	109	0.366
THL	297	117	0.394
WSL	306	141	0.461
Mean	297.5	116.88	0.392

CHL- Caohu Lake population; DTH - Dongting Lake population; EHL - Erhai Lake population, HLR - Heilong River population; HZL - Hongze Lake population; PYL - Poyang Lake population; THL - Taihu Lake population; WSL - Weishan Lake population

Table 4. Pairwise *Fst* values (below diagonal) and genetic distance (above diagonal) among eight northern snakehead populations in China based on AFLP data

Snakehead Population	CHL	DTL	ERL	HLR	HZL	PYL	THL	WSL
CHL		0.0349	0.115	0.113	0.0932	0.0334	0.0505	0.102
DTL	0.065*		0.104	0.0985	0.0816	0.0257	0.0555	0.0908
ERL	0.284*	0.273*		0.155	0.131	0.116	0.136	0.135
HLR	0.169*	0.146*	0.382*		0.115	0.106	0.124	0.112
HZL	0.119*	0.0914*	0.332*	0.162*		0.0598	0.0996	0.015
PYL	0.0468*	0.0344*	0.293*	0.154*	0.077*		0.036	0.0683
THL	0.083*	0.0584*	0.309*	0.183*	0.139*	0.0546*		0.111
WSL	0.131*	0.113*	0.335*	0.155*	0.0186*	0.103*	0.154*	

Note: * indicate significance ($p<0.05$)

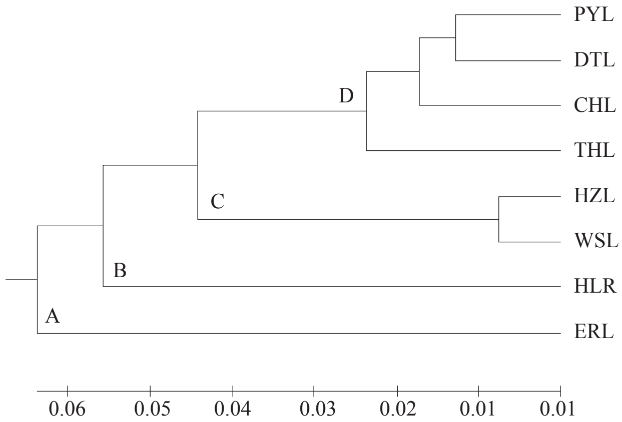

Fig. 1. UPGMA dendrogram of eight northern snakehead populations in China

Discussion

In this study, 240 specimens of northern snakehead were investigated representing eight populations in China. To date, there have been few data concerning genetic diversity of northern snakehead with AFLP markers. Thus, it can only be compared with genetic diversity of other fish species. The average proportion of polymorphic loci per population of northern snakehead was estimated as 0.392, ranging from 0.321 to 0.461, and the genetic diversity per population ranged from 0.092 to 0.128. Compared to other fish species, the northern snakehead population examined here exhibited a lower level of genetic diversity (Table 5). As it was the first attempt to elucidate the genetic structure of northern snakehead populations based on the AFLP marker and there was no historical data available on proportion of polymorphic loci and Nei′s genetic diversity for the *Channa* species, it would be difficult to explain why the northern population exhibited a lower level of genetic diversity. The most likely explanation may be summarised as follows. Firstly, the northern snakehead population was under stress due to deterioration in the environment, which led to less recruitment of the species and the loss of genetic diversity (Alam and Islam, 2005; So *et al*., 2006; Wang *et al*., 2010). The adverse habitat (such as adverse climate and ecological niche) may restrict the growth and variance of the fish species (Guo *et al*., 2010). Secondly, the natural resource of northern snakehead was imperiled due to overfishing. Overfishing has been a severe problem during the past decades in China (Xie and Chen, 1996; Fu *et al*., 2003; Wang and Zhang, 2008). This might have led to the reduction in effective population size and yield, and ultimately the loss of genetic diversity and population viability (So *et al*., 2006). Thirdly, the introgression of cultured stocks due to floods and improper management of pond farming might have resulted in reduction in genetic diversity of wild population. Wang *et al*. (2007) reported that huge floods may cause high gene flow between hatchery and wild populations due to the migration of

Table 5. Comparison of genetic parameters among selected fish species

Species	Proportion of polymorphic loci (%)	Nei′s genetic diversity	Reference
Channa argus	32.09 - 46.08	0.0920 - 0. 128	Present study
Pseudosciaena crocea	62.20 - 76.60	-	Wang *et al*. (2002)
Lutjanus argentimaculatus	41.89 -57.14	-	Zhang *et al*. (2004)
Nibea albiflora	51.70 - 51.99	0.0996 - 0.1022	Han *et al*. (2006)
Trachidermus fasciatus	50.00 - 50.28	0.2534 - 0.2592	Xu *et al*. (2008)
Brachymystax lenok	33.39 - 67.80	0.1446 - 0.2737	Wang *et al*. (2009)
Hucho taimen	43.64 - 55.27	0.148 - 0.195	Tong *et al*. (2009)
Anguilla japonica	62.81 - 74.10	0.2781 - 0.3077	Zhang *et al*. (2010)
Decapterus maruadsi	58.97 - 62.70	0.1809 - 0.1875	Zhang *et al*. (2010)
Cyprinus carpio L.	-	0.1036 - 0.1398	Zhong *et al*. (2010)
Chanos chanos	13.73 - 71.76	-	Adiputra *et al*. (2012)

farmed fish. Significant loss of genetic variation had been reported in hatchery populations/fish stocks (Allendorf and Phelps, 1980; Ryman and Stahl, 1980; Liao *et al*., 2006; Thai *et al*., 2007).

Clustering order always reflect relationship among populations. Results of the present investigation showed that Weishan Lake population and Hongze Lake population shared the highest genetic identity among the eight populations, indicating the closest genetic relationship. Populations of Heilong River and Erhai Lake showed the lowest genetic identity and their genetic relationship was the farthest.

The population structure of freshwater organisms is primarily dependent on the distribution of the river systems (Hara *et al*., 1998; Nagarajan *et al*., 2006; Wang *et al*., 2008). Structure of the eight northern snakehead populations also showed this close relationship. According to the UPGMA dendrogram, the eight populations can be divided into four clusters: A, B, C and D (Fig.1). Clusters A and B contain Erhai Lake and Heilong River populations, respectively. Erhai Lake belongs to the Lancang River basin, which is far away from the Heilong River basin. Cluster C contains the Weishan Lake and Hongze Lake populations, which belong to the Huaihe River basin. While cluster D contains the Caohu Lake, Dongting Lake, Poyang Lake and Taihu Llake populations, which belong to the Yangtze River basin. This suggested that the genetic differentiation was mainly because of the geographic proximity (Li *et al*., 2007).

Information on population genetic diversity is very important for genetic resource conservation and management. Northern snakehead hatchery owners often obtained brood population from the wild or from other fish hatcheries (Wu *et al*., 1999; Lei, 2009). The brood population usually comprise minimum number of brooders with unknown genetic background or limited genetic information. This type of poor brood population management would lead to further loss of genetic diversity and germplasm degradation, which may decrease the potential of species to persist in the face of abiotic and biotic environmental change (Allendorf and Ryman, 1987; Hammer and Teklu, 2008). In order to arrest loss of genetic diversity, hatcheries should be advised not to use fewer numbers of brood populations to meet production target. The genetic information of northern snakehead derived from the present investigation will provide guidelines to identify populations and categorise their population of origin to design reasonable management programmes for genetic resource conservation and genetic improvement by selective breeding.

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